TREATMENT OF INFECTED DENTAL PULPS OF MONKEYS WITH VANCOMYCIN AND HYALURONIDASE

By

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INTRODUCTION

Physiologic repair of the exposed dental pulp is the desired result of pharmacologic treatment. The major objectives in achieving repair is control and inhibition of infection and inflammation.

Numerous medications as well as techniques have been employed to reach these objectives. In recent years medications composed of one or more antibacterial agents such as antibiotics, sulfonamides, fungicides, corticosteroids or other ingredients have been popular. The most common mode of application is the direct placement of a specific agent on the exposed pulp. Most of these studies are clinical in nature. It is well established that clinical results often do not correlate well with histologic findings. Histologic studies have been inconclusive and require further investigation.

It is hoped that this study will add to our knowledge of direct pulp therapy with an antibiotic and an anti-inflammatory enzyme.

REVIEW OF THE LITERATURE

With the advent of penicillin therapy in 1942, much speculation had been made as to their use in dentistry. Kolmer,[1] for example, recognized the potential role of antibiotic compounds when he noted that intramuscular injections of penicillin may be helpful in the treatment of acute early pulpitis.

Bonner[2] reported the first clinical use of an antibiotic in pulp capping.* He mixed dry crystalline penicillin and glycerin into a paste and placed this over pulp exposures. Penicillin alone was used as the pulp capping agent in 113 human teeth, resulting in a clinical success of 100 per cent. The only criterion for selection of teeth for treatment was a pain response when the pulp was pierced by an explorer. Six teeth treated successfully with penicillin were re-opened and vital tissue was found.

Kutscher[3] treated vital carious exposures with penicillin sodium. He found that penicillin seemed to eliminate sepsis on the tooth surface and at the exposure site, and seemed to be absorbed throughout the pulp. One hundred and fifty-four teeth were treated with three failures recorded within four days. The treated teeth were observed at six weeks with 98 per cent success.

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*Pulp capping - the application of a medicament directly to the dental pulp.

The author concluded that the pulp can continue to function as normal tissue after exposure if the source of infection is eliminated, and if the resultant infection is controlled.

Gilberg,[4] in 1951, did not find age to be significant when he reported more than 97 per cent success when pulp capping with penicillin in patients ranging in age from two to 50 years. He used the penicillin in combination with zinc oxide and water as a base over a treatment of penicillin alone. Pain of 24 hours duration after this pulp capping procedure resulted in the tooth being considered a failure.

Burkman et al.[5] reported 75 per cent success in sterilizing carious dentin using indirect pulp capping with penicillin and camphorated monoparachloraphenol. In this experiment, no attempt was made to remove the deeper layers of decay. Treated carious dentin was removed from the teeth at a later date and cultured on eight different media. In 93 per cent of the teeth treated in this manner, no bacterial growth was obtained from carious dentine.

Ninety-four per cent clinical success was reported by Roberts[6] when he used intramuscular injections of penicillin in the treatment of exposed pulps. Calcium hydroxide was the medication placed over the exposed pulps.

Kutscher,[7] in 1953, reported the use of terramycin in combination with zinc oxide and eugenol and terramycin alone as a dressing on 20 pulp exposures. In all cases the medicament was considered an irritant because pain resulted when the dressing was applied. Relief was obtained by using anesthetics. No deleterious clinical results were recorded by the author over the three to 15 month period of investigation.

The first histologic study involving an antibiotic pulp capping agent was conducted on 18 teeth by Seelig et al.[8] They evaluated the efficacy of local antibiotic application in the treatment of infected dental pulps of Rhesus monkeys. A non sterile technique was used in which the coronal pulps were exposed and macerated with a bur. Penicillin G in a paste was forced into the preparations. The teeth were extracted after only seven days and the effect of the surgical procedures and antibiotic medication were studied microscopically. All of the pulps were evaluated as normal and demonstrated dentin bridge formation.

Amler[9] attempted to control pulpitis by systemic antibiotic therapy using buffered long acting penicillin and terramycin. In 16 cases in which therapy was initiated within two days of the onset of pain, 15 were clinically successful after 18 months.

In 1956, Morales[10] reported the use of streptomycin, penicillin, and sulfonamide as a pulp capping mixture for anterior teeth. In posterior teeth he recommended the use of chlortetracycline plus chloramphenicol as a pulp capping agent. Only four failures were reported among 150 teeth that were treated. The author attributed two failures to improper diagnosis and two failures to the breakdown and loss of the cement fillings.

Englander et al.[11] performed pulpotomies on 228 teeth in young adults. The pulp stumps were covered with one of the following: a polyantibiotic paste, tetracycline in various calcium salts, tetracycline in a non-calcium diluent, and penicillin powder. The average time prior to postoperative examination of treated teeth was 43 days. The polyantibiotic paste and tetracycline in various calcium salts demonstrated 100 per cent success. Tetracycline in a non-calcium diluent resulted in 82 per cent success and penicillin registered 64 per cent success.

James, Englander and Massler[12] treated 131 teeth with vital pulp exposures due to caries. In all instances there was a history of preoperative pain. The pulps were amputated and treated with various calcium compounds, antibiotics, or a combination of antibiotics and calcium compounds. Histologic evaluation revealed that bridging occurred more frequently under the calcium compounds than

under either the antibiotics alone or the antibiotics combined with calcium compounds.

Kiryati,[13] in 1958, used polyantibiotics in combination with hydrocortisone in pulp capping surgically exposed pulps of rat molars. He found the mixture to be superior to hydrocortisone alone. The author stated that the administration of hydrocortisone did not show great promise, even in combination with an antibiotic.

Seltzer and Bender[14] pulp capped surgically exposed dog teeth with an aqueous solution of 250,000 units of potassium penicillin. They found necrotic pulps and apical granulomas in each of the 52 teeth treated after periods of seven to 90 days. The authors stated that pulp capping is a questionable procedure, even under ideal conditions and that the formation of a dentin bridge is a dubious criterion for success.

In 1962, Schroeder and Triaden[15] relieved pain in 214 adult human teeth by pulp capping with a mixture of triamcinolone, chloramphenicol, xylocaine solution, and an ointment base. All of the treated teeth were pain free within two to three hours after treatment and at two to three weeks the treated teeth were asymptomatic and vital. Two teeth were extracted and studied histologically. One with the symptoms of pulpitis appeared to show signs of slight hyperemia and the other, which

was considered normal, showed no signs of inflammation when examined microscopically.

Kiryati[16] studied the effect of proteolytic enzymes on inflamed pulps in rat molars. Streptokinase and streptodornase did not effect the reparative process of the pulps; but when the enzymes were used in a mixture with cortisone and oxytetracycline the greatest success was recorded.

Vigg,[17] in 1962, reported in a clinical study that after 20 months he was still having success in almost all instances in the 66 teeth he treated with an experimental drug combination. The drug consisted of one per cent hydrocortisone and three per cent oxytetracycline in an ointment base. Lack of radiographic evidence of pathology and a lack of pain were his criteria for success.

Fiore-Donno and Baume[18] studied histologically 123 human pulps which had been capped with corticosteroid containing compounds. The authors recommended caution when using these compounds. In this study they found a lack of formation of a dentin barrier at the amputation site, even after long periods of time, and believed that these "open" pulps become increasingly susceptible to reinfection as the sealing ability of the filling material decreased with age. Many of these teeth were judged clinically successful but were judged unsuccessful his-

tologically. It was stated that glucocorticosteroids engender in pulpal tissue fibrotic metaplasia while they inhibit the collagenic activity of ordinary connective tissues.

Talim[19] reported that terramycin produces an area of chronic inflammation when used as a pulp capping agent on amputated pulps and retards dentin bridge formation. Sixteen of 31 treated human teeth were used as controls and received calcium hydroxide as a medication. The remaining teeth were treated with terramycin. All teeth were extracted within seven to 60 days after treatment and prepared for histologic examination. Dentin bridge formation was found under all medications, but the bridge formation was located further away from the amputation site in the antibiotic treated teeth than in the control teeth.

Gardner and his co-workers[20] investigated histologically the effect of Neosporin* (Polymixin B, Neomycin, and Bacitracin) on surgically exposed teeth in dogs. After two or three month periods, 27 teeth were extracted and prepared for histologic evaluation. Twenty-four showed indications of partial pulpitis and the remainder, further degenerative changes.

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*Neosporin, Burroughs, Wellcome and Company Incorporated, Tuckahoe, New York

Antibacterial agents were tested in exposed dental pulps of rats by Burke and Knighton.[21] The pulps were exposed surgically and an antibacterial agent in combination with zinc oxide and eugenol was placed over the exposures. The animals were then given intravenous injections of Staphylococcus aureus to produce bacteremias. Cultures were taken from the pulps at one and seven day intervals and placed in culture media. When penicillin was used in the pulp capping agent bacterial growth appeared in 47.5 per cent of the specimens at one day and in 6.9 per cent at seven days. Bacitracin‡ when used in the pulp capping agent resulted in bacterial growths in 50.9 per cent of the media but at seven days no growths were recorded. The other antibacterial agents ranged from 84.4 per cent to 93.2 per cent bacterial colony formation at one day and 71.4 per cent to 96.6 per cent at seven days.

In 1962, Gasan-Zade[22] reported more than 90 per cent clinical success using antibiotics to treat pulpitis. His technique involved removing caries on the first visit and placing an ointment of chlortetracycline on the cavity floor. If pain persisted after this treatment a local anesthetic was administered in combination with 100,000 units of penicillin. At two days the dressing

‡Bacitracin, Eli Lilly and Company, Indianapolis, Indiana

over the pulp was removed and a new application of chlortetracycline and 20 per cent oil of camphor was placed.

In 1964, Lawson and Mitchell,[23] using double blind control procedures in a clinical study, tested antibiotics in combination with a corticoid in treating 27 adult teeth with painful pulpitis. No failures were experienced upon re-examination after an average of 91 days. The experimental drug combination (erythromycin estolate, streptomycin sulfate, and flurandrenolone) was considered to have definite therapeutic value when compared to the starch treated control teeth which experienced about 50 per cent failures. Seven teeth were studied histologically in this study. The control teeth showed persistent pulpitis and the corticoid antibiotic treated teeth revealed more normal appearing pulps. The authors concluded: painful pulpitis seems to be a reversible process when treated; painless pulpitis can occur without clinical symptoms; and histologic interpretations do not necessarily correlate with clinical observations.

Mager[24] treated pulpitis with a synthetic steroid combined with antibiotics.§ In 20 cases he found that all of the teeth treated except one had retained their vitality when examined after six months. Relief of pain was

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§ Ledermix Dental Compound

immediate after initial treatment and in all cases no side effects were observed.

In a clinical study, Olsen[25] reported promising results with the use of a corticoid and demethylchlortetracycline compound" in 370 cases of hyperemia, pulpitis, and apical periodontitis. He found that pain was usually relieved within 30 minutes, and at follow-up examinations after six to 12 months only three teeth had become non-vital.

Ehrman,[26] in 1965, reported that 22 teeth with symptoms of acute suppurative pulpitis were treated with Ledermix "A" and "B." Sixteen of these teeth tested non-vital when examined at six months, but most of them were free of pain. More success was recorded when treating hyperemia or early pulpitis than that experienced in the more advanced forms of pulpal pathology.

Haldi and John[27] used sulfonamide administered subcutaneously and penicillin G injected intravenously to observe pulp fluid levels of these drugs. They found that these drugs used in this manner appeared in the dental pulp fluid in approximately the same concentration as in the blood.

In 1966, Schneider and Lawson[28] compared a pulp capping mixture of a corticoid and an antibiotic with

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"Ledermix Dental Compound

calcium hydroxide in a double blind controlled study involving 58 human teeth. The corticoid-antibiotic mixture met with 76 per cent clinical success, and the calcium hydroxide resulted in 90 per cent success. Histologic examinations revealed slightly more inflammation in the teeth treated with the corticoid-antibiotic mixture than in those treated with the calcium hydroxide.

The treatment of dental pulps with a mixture of antibiotics and hyaluronidase was investigated in a clinical study by Janiszewska.[29] Vital teeth with toothaches and deep carious lesions were excavated. All carious dentin was removed unless the procedure was too painful to the patient, in which cases some carious dentin was left on the floor of the cavity. The paste consisting of Declomycin,# erythromycin, kanamycin, and hyaluronidase was placed over the cavity floor and sealed in with zinc oxide. Forty-one cases were treated in this manner and re-examined over a period of 12 months. Toothaches returned in four of these treated teeth, but the teeth remained vital. The author recognized the need for a histologic and long term study using the pulp capping agent containing hyaluronidase to substantiate the 90 per cent clinical success reported in this study.

#Declomycin, Lederle Laboratories (A Division of American Cyanamid Company), Pearl River, New York

Mullaney, Lawson and Mitchell[30] studied the same medications and many of the same teeth in a follow-up of the preliminary report of Lawson and Mitchell.[23] Originally 100 per cent success was reported after about three months in the clinical portion of this study using a corticoid-antibiotic mixture in the treatment of painful pulpitis. The average time for re-examination of the treated teeth was extended to two years four months and 10 days. A significant decrease in success was observed at this longer recall date. The corticoid-antibiotic mixture resulted in 71.4 per cent success.

In another part of this study, 28 monkey pulps were exposed surgically and treated. One-half of the sample served as controls. Twelve teeth were extracted at 90 days and the remainder were removed at 180 days. Histologic examination of these teeth revealed that all were normal except two control teeth which had abscesses and marked inflammation. The main difference observed was that the experimental material seemed to allow for more extensive dentinoid bridge formation. Also, less inflammation was apparent below the bridging in the corticoid-antibiotic treated teeth.

Clinical observations indicated that painful pulpitis is reversible and the experimental drug is of value in treating it. The animal portion of this study re-

vealed a slight advantage of the corticoid-antibiotic material over the control material.

In 1966, Fiore-Donno and Baume[31] concluded in a clinical and histological study that the use of corticosteroid pastes in direct pulp capping is contraindicated. In reviewing 30 pulps with a history of spontaneous pain prior to treatment, the investigators found histologically that there was an arrestment of dentinogenesis at the exposure site, bridging never occurred, and as the postoperative period lengthened, more residual chronic inflammation occurred.

Weine[32] used a fungicide in combination with oxytetracycline as a pulp capping agent. He treated 57 teeth in a clinical study and had 89.3 per cent success with the antibiotic-fungicide combination and 96 per cent success with the oxytetracycline alone. These teeth were tested clinically with electrical pulp testers. Radiographs were made and clinical symptoms were recorded. A histological study was initiated to evaluate microscopically the effect of these drugs on the pulp. The author concluded that oxytetracycline is more effective as a pulp capping agent than is the mixture of oxytetracycline and nystatin.

Baker[33] using a modified double blind controlled technique studied the effect of two antibiotics in combi-

nation, streptomycin sulfate and erythromycin estolate, and a control of starch when pulp capping surgically exposed and intentionally infected teeth in monkeys. One-half of the 52 teeth used in this study were extracted at 30 days and the remainder were removed at 90 days. Histologic evaluation revealed that varying degrees of inflammation were still present in all of the teeth, but those pulp capped with the antibiotic compound had the least.

In 1968, Compton[34] again evaluated 18 of the teeth that were initially studied by Lawson and Mitchell[23] in 1964 and re-evaluated by Mullaney, Lawson and Mitchell[30] in 1966. Thirteen of the teeth were treated with erythromycin estolate, streptomycin sulfate, and flurandrenolone, and the remainder were controls treated with starch. Six of the teeth successfully treated with the corticoid-antibiotic mixture were evaluated as still clinically successful after five years. All of the control teeth were still clinically successful. The author concluded by stating that painful pulpitis in permanent teeth of adults is reversible. The corticoid-antibiotic mixture did not seem to be superior to the starch control over the long term of this study. Also, he emphasized the lack of correlation between clinical and histologic findings.

A number of other studies[35-49] have been reported but

will not be included in this review. While these authors demonstrated interest in the treatment of dental pulps with antibiotics, their studies lacked controls, were of a short duration, or included many variables. Also, most of these studies were of a clinical nature only, supplied little data, involved only a few teeth, or did not include histologic evidence. For these reasons their results could not be accepted with any confidence.

STATEMENT OF THE PROBLEM

This study was undertaken to histologically evaluate the effect of a pulp capping agent on exposed and infected dental pulps of the Macaca Speciosa monkey. The agent consisted of a combination of an antibiotic, vancomycin hydrochloride, and an anti-inflammatory enzyme, hyaluronidase.

A hypothesis of this study is that the monkeys will respond to the experimental medications in a manner similar to man.

EXPERIMENTAL PROCEDURE

Formulation of Medicaments

The drugs used in this investigation were produced by Eli Lilly and Company and the G.D. Searle and Company. The following list of powder and liquid combinations represent the four experimental medicaments and controls used in this study:

1. Powder: vancomycin hydrochloride (Vancocin^{R})* 10%
   starch q.s.
   Liquid: U.S.P. water
2. Powder: vancomycin hydrochloride (Vancocin^{R}) 10%
   starch q.s.
   Liquid: hyaluronidase (Alidase^{R})+ 150 U.S.P. units per 1 cc. of U.S.P. water
3. Powder: starch q.s.
   Liquid: U.S.P. water
4. Powder: starch q.s.
   Liquid: hyaluronidase (Alidase^{R}) 150 U.S.P. units per 1 cc. of U.S.P. water

Sufficient powder was mixed with one or two drops of the liquid to produce a thick creamy paste.

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*Vancocin^{R}, Eli Lilly and Company, Indianapolis, Indiana
+Alidase^{R}, G.D. Searle and Company, Chicago, Illinois

PRELIMINARY STUDIES

Antibiotic Inactivation Test

The compatibility of the drugs to be used in combination as pulp capping agents was tested by inoculating plates of both trypticase soy agar and trypticase soy-human blood agar with 13 different organisms recently isolated from oral infections in human patients. Each inoculated plate received four separate smears, one of each experimental medicament. The plates were observed at 48 and 72 hours and those with zones of inhibition were recorded as sensitive.

Tissue Compatibility Test

Materials for subdermal implants were prepared by mixing the various drug combinations into a paste similar to that to be used throughout this study to cover exposed pulps. The pastes were then placed into molds and allowed to dry. Pellets approximately 1.5 by 2.0 millimeters were then separated from the molds.

Six healthy adult Wistar rats with weights ranging from 245 to 310 grams were prepared and operated in the following manner:

1. Anesthesia was induced by peritoneal injections of a 10 per cent solution of Somnopentyl. Each animal receiving 0.70 cubic centimeters per 100 grams of body weight.

2. The animals were then given identifying marks in their ears by punching holes with a rubber dam punch.
3. Animal electric shears were used to shave the dorsal area of the animals from the neck to the pelvis. Gauze squares saturated with 70 per cent alcohol were used to clean the shaved areas.
4. Four horizontal incisions were made using a Bard-Parker #5 blade. These incisions were approximately one centimeter in length and were located in the dorsal interscapular, the dorsal pelvic, and two areas in between (Figure 1).
5. A test pellet was placed in the subdermal tissue approximately five millimeters cephalad to each incision. Each animal received four test pellets, one of each of the drug combinations.
6. One silk suture was used to close each incision.
7. The animals were then returned to their cages and observed. Within one hour all became active and satisfactorily recovered from the effects of surgery and anesthesia. The animals were again observed at 24 hours and all appeared normal and all sutures were in place.
8. Two animals were sacrificed at two days, at 16 days, and at 32 days by placing them in a closed jar containing towels saturated with ether.

9. Animal electric shears were used to remove the hair on the 16 and 32 day animals in the same manner as described previously.
10. A Bard-Parker #5 blade was used to remove a section of tissue including each implant. Each excised piece of tissue measured approximately 2.5 by 3.5 centimeters and was immediately placed in a 10 per cent formalin solution for initial fixation.

Later the specimens were trimmed through the implants, embedded in paraffin, and seven microns sections were cut and stained with hematoxylin and eosin. Microscopic evaluations were made of semi-serial sections through various areas of each implant and surrounding tissues.

Perfection of Surgical Technique and Bacterial Contamination of 24 Hour Pulp Exposures

A stump-tailed macaque monkey (Macaca Speciosa) was prepared for surgery in the following manner:

1. The animal was removed from its cage with the aid of a net and weighed.
2. An intraperitoneal injection of nembutal sodium was given using a 20 gauge short needle. The recommended dosage of 1.00 ml. (60 mg.) for every three pounds of body weight was used.

3. When profound anesthesia was achieved, radiographs of the four maxillary bicuspids were taken and developed.
4. The animal was then placed in a supine position on the operating table with its head tilted back. No difficulty was experienced in maintaining an unobstructed airway.
5. A careful clinical and radiographic examination was made and any pathologic or unusual conditions and the morphology of the dental pulps were noted.
6. Class V cavities were prepared in the four maxillary bicuspids using a #57 friction grip bur rotating at high speed without water. No attempt was made to operate under aseptic conditions.
7. A well was then made in the cavity floor using a #2 bur at slow speed. Compressed air was used to remove dentin chips and debris as the cavities were being prepared.
8. The cavities were irrigated with water and dried with air.
9. A #1 Jacquette scaler was used to expose the dental pulps by applying firm pressure with the point of the instrument to the thin dentin floor.

10. The pulp was exposed by stabbing with an explorer until hemorrhage appeared. Care was taken to avoid deep penetration of the explorer into the coronal pulp. Saliva from the vestibular area and tongue was carried to the exposures to aid in contamination. The exposures were left open to the oral environment.
11. The animal was then returned to its cage and observed for postsurgical complications.

Twenty-four hours later the animal was again prepared for the following surgical procedures.

1. The four maxillary bicuspids were extracted using a straight elevator to free the attached gingiva from the teeth and a pedodontic forcep.
2. The extracted teeth were immediately placed in a 10 per cent formalin solution for initial fixation.
3. The animal was returned to its cage and was observed for postsurgical complications.
4. The extracted teeth were trimmed from the mesial aspect with a #57 friction grip bur rotating at high speed. The pulps were approximated by this trimming with care taken to avoid penetration of the pulp chamber. The teeth were returned to the formalin for further fixation.

Specimens were decalcified and seven microns thick serial paraffin sections were made through the exposure sites. Staining was done with hematoxylin and eosin on alternate sections and the Brown and Brenn[50] stain was used on selected sections.

PRINCIPAL STUDY

This portion of the investigation was originally designed for a modified double blind control testing procedure. Preliminary studies familiarized the author with the test agents' physical properties. The antibiotic containing agents were tan rather than yellow in color and were of a more coarse consistency than the non-antibiotic agents. Therefore, to eliminate bias each tooth was designated a specific medicament by using a random number table and double blind control procedures were initiated when the author evaluated the histologic sections.

Surgical Procedures

Two Macaca Speciosa monkeys were prepared for surgery as described in the preliminary study. All surgical techniques were the same except that full mouth radiographs were taken, 28 teeth in each animal were operated, and an explorer was used rather than a #1 Jacquette scaler to make the initial pulp exposures (Figure 2).

Twenty-four hours later the animals were prepared for surgery as described and the following procedures were carried out:

1. A visual examination was made.

2. A small spoon excavator was used to remove debris from the cavity preparations. A blood clot which had formed at each exposure site was teased away with an explorer.
3. Saliva from the vestibular area was carried to the exposure areas and mixed with the hemorrhage or fluid at the exposure site in an attempt at further contamination.
4. The cavities were then irrigated with water and dried with dry cotton pellets and light air pressure.
5. The selection of the medicament to be placed over each exposure was predetermined. The pulp capping agents were placed in the cavity preparations with the blunt end of a TP#3 plastic instrument and a pumping action was used in an attempt to force some of the agent into the pulp chamber.
6. After the pulp capping agents had dried, excess medicament was removed from the cavity preparation with the aid of a spoon excavator and light air pressure. An attempt was made to leave the medication in that portion of the preparation prepared by the #2 bur.

7. Zinc oxide and eugenol with four per cent zinc acetate crystals was placed as a protective base over the pulp capping agents.
8. A #57 bur rotating at slow speed was used to remove the excess zinc oxide and eugenol from the walls of the preparations and the field was cleared with light air pressure.
9. Silver amalgam was condensed into the cavity preparations and carved to conform to the natural anatomy of the tooth.
10. The animals were returned to their cages and were observed for postsurgical complications.

At 14 days the animals were removed from their cages with a net for a visual examination without the aid of an anesthetic. All restorations were intact and no visual signs of oral pathology were observed. This examination was repeated at 21 days and at 60 days in the 90 day animal.

At 30 days and 90 days an animal was prepared for surgery in the described manner and the following procedures were accomplished:

1. A visual oral examination was made with the aid of a mouth mirror, explorer, and adequate light.
2. Full mouth radiographs were made. These radiographs were inspected for pathology and root formation prior to surgical procedures.

3. A straight elevator was used to free the attached gingiva from the teeth.
4. Vertical incisions were made with a #15 Bard-Parker blade to facilitate raising of a labial or buccal mucoperiosteal flap with a #7 wax spatula.
5. The buccal and labial bone was then removed with the aid of a Dudley bone impactor and round burs rotating at high speed.
6. The teeth were removed with a pedodontic forcep and immediately placed in separate labeled bottles containing 10 per cent formalin.
7. The animals were then sacrificed.
8. The teeth were trimmed as described in the preliminary study and were immediately returned to the 10 per cent formalin solution for further fixation.

As in the preliminary study, a laboratory technician performed the procedures of preparing seven microns serial sections through the exposure sites of the specimens and staining with hematoxylin and eosin and Brown and Brenn stains.

Histologic Evaluation

Alternate slides of the seven micron serial sections through the exposures stained with hematoxylin and eosin

were evaluated using a modified double blind control. Pulp pathology such as necrosis, abscess formation, and inflammation were recorded as unsatisfactory responses. The quantity of reparative dentin was recorded. This interpretation was based on an empirical judgement by the author. All stained serial sections were evaluated two times. Interpretations of each evaluation were compared and any variations in the findings of the same specimens were evaluated a third time.

Eight previously unstained sections of teeth were selected for Brown and Brenn staining. These sections were stained in this manner to demonstrate by histologic means the presence or absence of bacteria in the dental pulp. The limited number of specimens stained reflects the tedious and lengthy staining procedures involved in this technique.

DATA

PRELIMINARY STUDIES

Antibiotic Inactivation Test

Pulp capping agents containing vancomycin, starch and water and vancomycin starch and hyaluronidase were effective in inhibiting growth of the gram-positive organisms tested, and ineffective in preventing growth of the gram-negative organisms. The control agents, starch and water and starch and hyaluronidase, did not inhibit any of the test organisms. No differences were observed in the size of the zones of inhibition produced by the antibiotic in combination with the hyaluronidase and the antibiotic in combination with water (Table I).

Tissue Compatability Test

Two day implant specimens of the four pulp capping agents were easily located at the time of sacrifice of the rats and all were observed in the histologic sections. In the 16 day animals, four implants could not be found by gross observation at the time of sacrifice. Serial histologic sections through the area where the implants were believed to have been placed revealed two of them. One of the gross observations of a 16 day specimen appeared to be an implant, but histologic examination of serial sections through this area revealed normal tissue with no implant present.

Five of the 32 day specimens could not be found by gross observation at the time of sacrifice. All but one were found by serial histologic examination of the area. Five of the 24 implants were not found in histologic sections; therefore, no responses could be recorded for them.

Subcutaneous connective tissue implants of the two test agents and the two controls lacked any irritation produced by the medicaments. All gross and microscopic responses were mild. The mild histologic responses were characterized by a thin fibrin capsule surrounding the implants and a general lack of inflammatory cells in the area (Table II, Figures 3, 4 and 5).

Bacterial Contamination of 24 Hour Pulp Exposures

Histologic sections of the four maxillary bicuspids stained by the Brown and Brenn technique demonstrated the presence of bacteria in the cavity preparations and in the coronal pulp in the immediate area of the exposure. Bacteria could not be demonstrated to have penetrated deeper.

Microorganisms could not be distinguished from the cellular elements of these pulps when the sections were stained with hematoxylin and eosin. These sections did demonstrate dentin chips in the exposure areas and the overall appearance of the pulps was normal.

PRINCIPAL STUDY

Clinical and Radiographic Findings

Clinical oral examinations were given each animal prior to surgery and at 14 days. In the 90 day animal, examinations also were made at 21 days and 60 days. No visual signs of pathologic conditions were observed during any of these examinations. All restorations remained intact throughout this study and were not removed until the teeth were decalcified prior to sectioning.

Radiographic examinations were made prior to surgery the day the pulps were exposed and the day the teeth were extracted. No apical pathosis or other unusual observations were recorded.

At the time the animals were sacrificed, buccal or labial mucoperiosteal flaps exposed the alveolar bone. No perforations of this bone or other pathologic conditions were noted.

Criteria for Histologic Evaluation of the Pulp

A satisfactory response by the pulp to the experimental procedures was demonstrated by the following histologic characteristics:

1. An apparent attempt at sealing off the exposure site from the remainder of the pulp by a reparative dentin bridge. This dentin was of an irreg-

ular tubular structure, was lighter staining than normal dentin, and appeared to be composed of fused reparative dentin from both the canal walls and chips. In no instance was a bridge observed to completely seal off the exposure site. Reparative dentin formation in these cases ranged from a moderate to a large amount.

2. Two zones were observed on the exposure side of the bridges. One, nearest the bridge, was a vascular area infiltrated with a small number of inflammatory cells. This zone blended into the second zone which was composed of a mixture of nuclei of once living inflammatory cells and experimental medication.
3. On the pulpal side of the reparative dentin bridge, inflammatory cells were rarely observed in the area adjacent to the bridge. The remainder of the pulp appeared normal. This pulp was considered to have had a favorable prognosis prior to extraction.

An unsatisfactory response of the pulp to the experimental procedures was denoted by the following histological characteristics:

1. Reparative dentin formation varied from a moderate amount to none at all. When present it was characterized by an irregular tubular structure, both on the chips and walls, but a definite lack of coalescence was observed. This lack of fusion indicated a comparatively ineffective attempt at sealing off the exposure area.
2. Many inflammatory cells were observed in the area of the exposure as well as a more widespread diffusion of these cells throughout the coronal pulp in many specimens.
3. Abscess formation in the area of the exposure was noted in some of these pulps.
4. In one specimen a chronic inflammatory type response resulted in the walling off of vital tissue in the apical one-half of the canal by a pyogenic membrane. The coronal one-half of this pulp was necrotic.
5. A favorable prognosis for these pulps was doubtful.

Necrosis indicated that all of the pulp had degenerated as a result of the experimental procedures. Recovery was not believed possible.

Histologic Response of the Teeth Treated with Vancomycin, Starch, and Hyaluronidase

At 30 days, seven of the teeth appeared to be recovering in a satisfactory manner (Table III). One tooth did not exhibit quite the amount of reparative dentin that the others did, but this tooth did demonstrate a good attempt at bridging. An unsatisfactory response occurred in one tooth. Reparative dentin was minimal around the chips and an area which may have been an abscess between the dentin chips and the exposure site (Figure 6) made the prognosis of this tooth doubtful. There was a lack of inflammatory cells in the tissue adjacent to the suspicious area.

All of the 90 day specimens responded with large amounts of reparative dentin, even the tooth exposed with a #2 bur. The response of these teeth was satisfactory. One unusual result did occur, the reparative dentin appeared to have "grown out" into the cavity preparation in its attempt to seal off the exposure (Figure 8).

Histologic Response of the Teeth Treated with Vancomycin, Starch and Water

Three of the teeth treated for 30 days responded in a satisfactory manner and four in an unsatisfactory manner (Table IV). One of the unsatisfactory responses exhibited no secondary dentin and abscess formation in

the coronal pulp (Figure 9). Reparative dentin did form in the other three unsatisfactory teeth, but it lacked coalescence and numerous inflammatory type cells were present in the immediate area of the dentin chips. The remainder of these pulps were normal.

Satisfactory responses were observed in all of the teeth treated for 90 days. Large amounts of reparative dentin and good bridge formation was present in all cases. One tooth exhibited the apparent growth of reparative dentin out into the cavity preparation similar to that observed in a tooth treated with vancomycin, starch, and hyaluronidase (Figure 8).

Histologic Response of the Teeth Treated with Starch and Hyaluronidase

Two of the starch and hyaluronidase treated control teeth responded in a satisfactory manner at 30 days. Four of the pulps treated for this period of time were completely necrotic (Figure 11) and another exhibited an unsatisfactory response (Figure 6). No reparative dentin had formed around the chips in the unsatisfactory tooth and inflammatory cells were present around these chips (Table V).

Only two of the teeth treated for 90 days were necrotic. Four responded in a satisfactory manner and

one was unsatisfactory. The latter had a minimal amount of reparative dentin around the chips and inflammatory cells were present in the area of the exposure.

Histologic Response of the Teeth Treated with Starch and Water

Three of the teeth treated for 30 days with this control medication were necrotic. Two recovered in a satisfactory manner and two in an unsatisfactory manner (Table VI).

Among the teeth treated for 90 days, four responded in a satisfactory manner. One of these had moderate reparative dentin formation and the other three had large amounts. Two other 90 day teeth were necrotic and one other was unsatisfactory in its response to the experimental procedures. Very little reparative dentin had developed in this tooth and inflammatory cells were scattered throughout the exposure area.

Histologic Evaluation of the Selected Sections Stained with Brown and Brenn

Seven slides of serial sections were selected for Brown and Brenn staining to demonstrate the presence or absence of bacteria in the dental pulps. These slides were selected for staining after all hematoxylin and eosin stained sections had been evaluated. Three of

these slides were of teeth which responded unsatisfactorily and four were of satisfactory responding teeth.

Bacteria were not present in any of the histologic sections of the pulps of satisfactory responding teeth and of one of the teeth which gave an unsatisfactory response. The presence of bacteria was evident in the two remaining unsatisfactory responding pulps. Large colonies of bacteria were observed in one canal extending down toward the apical area to a pyogenic membrane which separated the apical vital pulp from the coronal necrotic pulp where the bacteria were located (Figures 13 and 14). In the other pulp in which bacteria were found, the organisms were located in the immediate area of a coronal abscess, but no bacteria were found in the rest of the pulp. Of interest in these sections was the penetration of the dark staining starch granules into the pulp (Figure 10).

Statistical Analysis of Data

In order to test statistically for differences between teeth treated with each medicament, those teeth evaluated with small, moderate, or large amounts of reparative dentin formation were considered positive and those teeth with no reparative dentin formation negative. Teeth with satisfactory pulp responses were

considered positive pulp reactions and those with unsatisfactory reactions or necrosis were unsatisfactory.

These binomial data were first transformed using the Freeman-Tukey Angular transformation.[51] The transformed data were then tested for differences between 30 and 90 day postoperative reactions as well as differences between pulp capping agents using the analysis of variance (Table IX).

Results showed that for both dentin formation and pulp reaction, the four pulp capping agents resulted in responses that were significantly different from each other. With respect to dentin formation, 30 and 90 days postoperative did not differ, i.e., at 90 days, the number of cases of dentin formation did not increase significantly. With respect to pulp reaction, teeth treated for 30 and 90 days showed a difference, i.e., at 90 days, instances of satisfactory pulp reaction increased significantly over those of 30 days.

The absence of a combination effect of agent and time, implies that the difference in the percentages of positive responding teeth in 30 days and in 90 days remains the same regardless of the pulp capping agent used. Therefore the data for the two time periods were pooled to test for differences between any two groups, using the normal deviate test (Tables X and XI).

Results of individual comparisons between any two agents showed a significant increase in the percentage of favorably treated teeth in Group I (vancomycin, starch, and water) and in Group II (vancomycin, starch, and hyaluronidase) over the teeth in Groups III (starch and water) and Group IV (starch and hyaluronidase), in both dentin formation and pulp reaction.

Teeth treated with vancomycin, starch, and water (Group I) and vancomycin, starch, and hyaluronidase (Group II) showed no difference in percentage of teeth with dentin formation. However, with respect to pulp reaction, Group II treated with vancomycin, starch, and hyaluronidase showed significantly more favorable responses than Group I treated with vancomycin, starch, and water.

TABLES AND ILLUSTRATIONS

TABLE I

Results of Antibiotic Inactivation Test

Organism	Type of Agar	Gram Stain	Response to: Vancomycin Starch Hyaluronidase	Vancomycin Starch Water
Providence	TSA	Neg	R	R
Proteus Marganii	TSA	Neg	R	R
Escherichia Coli	TSA	Neg	R	R
Aerobacter	TSA	Neg	R	R
Herellea	TSA	Neg	R	R
Pseudomonas	TSA	Neg	R	R
Proteus Mirabilis	TSA	Neg	R	R
Pneumococcus	TSAHB	Pos	S	S
Beta Streptococcus	TSAHB	Pos	S	S
Staphylococcus Coagulase Negative*	TSAHB	Pos	S	S
Staphylococcus Coagulase Negative*	TSA	Pos	S	S
Staphylococcus Coagulase Negative*	TSA	Pos	S	S
Staphylococcus Aureus	TSA	Pos	S	S

*These organisms were isolated from different patients.

None of the organisms were inhibited by the control starch and water and starch and hyaluronidase mixtures.

TSA - Trypticase soy agar
TSAHB - Trypticase soy-human blood agar
Neg - Gram negative
Pos - Gram positive
R - Resistant
S - Sensitive

TABLE II

Histologic Response to the 24 Implants in the Subcutaneous Connective Tissue of Rats

Slide Series Number	Time in Days	Implant Material	Implant Found Clinically	Histologic Response
7814	2	SH	Yes	Mild
7815	2	VSH	Yes	Mild
7816	2	VSW	Yes	Mild
7817	2	SW	Yes	Mild
7818	2	SH	Yes	Mild
7819	2	VSH	Yes	Mild
7820	2	VSW	Yes	Mild
7821	2	SW	Yes	Mild
7857	16	SH	No	Mild
7858	16	VSH	No	
7859	16	VSW	Yes	Mild
7860	16	SW	Yes	Mild
7861	16	SH	No	
7862	16	VSH	Yes	
7863	16	VSW	Yes	Mild
7864	16	SW	No	
7944	32	SH	No	Mild
7945	32	VSH	No	Mild
7946	32	VSW	No	Mild
7947	32	SW	Yes	Mild
7940	32	SH	Yes	Mild
7941	32	VSH	No	
7942	32	VSW	Yes	Mild
7943	32	SW	No	Mild

VSH - Vancomycin, starch and hyaluronidase

VSW - Vancomycin, starch and water

SH - Starch and hyaluronidase

SW - Starch and water

Preface to Tables III, IV, V, VI, VII and VIII

Key to Headings

Slide Series Number - laboratory technician's identifying number given to each group of serial sections of a tooth.

Tooth Number - tooth number according to the universal numbering system.

Method of Exposure - indicates the method used to make the initial penetration into the pulp chamber.

Pulp Capping Agent - indicates the medicament used to cap an exposure.

Reaction of Pulp - indicates the response of the dental pulp to experimental procedures.

Symbols

Exp - indicates the point of an explorer was used to make the initial pulp exposure.

#2 - indicates a #2 bur rotating at slow speed made the initial exposure.

#2F - indicates a #2 bur rotating at slow speed penetrated into the pulp chamber.

1J - indicates a #1 Jacquette scaler was used to make the initial pulp exposure.

VSW - Vancomycin hydrochloride 10% and starch q.s. mixed with U.S.P. water.

VSH - Vancomycin hydrochloride 10% and starch q.s. mixed hyaluronidase 150 U.S.P. units per 1 cc. of U.S.P. water.

SW - Starch mixed with U.S.P. water.

SH - Starch mixed with hyaluronidase 150 U.S.P. units per 1 cc. of U.S.P. water.

Lge - large amounts of reparative dentin on canal walls and around dentin chips with an apparent attempt at the formation of a complete bridge.

Mod - moderate amount of reparative dentin on canal walls and around dentin chips.

Sml - small amount of reparative dentin on canal walls and around dentin chips.

None - no reparative dentin formation present.

Sat - indicates a satisfactory response of the pulp to the experimental procedures.

USat - indicates an unsatisfactory response of the pulp to the experimental procedures.

Nec - indicates that all or the major part of the pulp has become necrotic due to the experimental procedures.

TABLE III

Histologic Findings in the 14 Teeth Treated with the Antibiotic in Combination with Hyaluronidase

Slide Series Number	Tooth Number	Method of Exposure	Reparative Dentin Formation	Reaction of Pulp
30 Days Postoperative				
7982	5	Exp	Mod	Sat
7983	21	Exp	Lge	Sat
8003	7	Exp	Lge	Sat
8006	10	Exp	Lge	Sat
8010	14	Exp	Sml	USat
8014	20	Exp	Lge	Sat
8015	22	Exp	Lge	Sat
90 Days Postoperative				
8023	2	#2	Lge	Sat
8038	18	Exp	Lge	Sat
8042	23	Exp	Lge	Sat
8043	24	Exp	Lge	Sat
8046	27	Exp	Lge	Sat
8048	29	1J	Lge	Sat
8050	31	Exp	Lge	Sat

TABLE IV

Histologic Findings in the 14 Teeth Treated with Antibiotic Compound Alone

Slide Series Number	Tooth Number	Method of Exposure	Reparative Dentin Formation	Reaction of Pulp
30 Days Postoperative				
8000	3	2F	Mod	USat
8001	4	Exp	Mod	USat
8004	8	Exp	Lge	Sat
8007	11	Exp	Lge	Sat
8009	13	#2	Sml	USat
8017	25	Exp	None	USat
8022	31	Exp	Lge	Sat
90 Days Postoperative				
8025	4	1J	Lge	Sat
8027	6	#2	Lge	Sat
8029	8	Exp	Lge	Sat
8031	10	#2	Lge	Sat
8034	13	Exp	Lge	Sat
8039	20	Exp	Lge	Sat
8049	30	Exp	Lge	Sat

TABLE V

Histologic Findings in the 14 Teeth Treated with the Starch and Hyaluronidase Control

Slide Series Number	Tooth Number	Method of Exposure	Reparative Dentin Formation	Reaction of Pulp
30 Days Postoperative				
7984	24	Exp	None	Nec
8002	6	Exp	Lge	Sat
8008	12	Exp	None	USat
8011	15	Exp	None	Nec
8013	19	Exp	Mod	Sat
8016	23	Exp	None	Nec
8020	29	Exp	None	Nec
90 Days Postoperative				
8028	7	Exp	None	Nec
8035	14	Exp	Sml	USat
8036	15	#2	None	Nec
8040	21	Exp	Lge	Sat
8041	22	Exp	Lge	Sat
8044	25	Exp	Lge	Sat
8047	28	1J	Lge	Sat

TABLE VI

Histologic Findings in the 14 Teeth Treated with the Starch and Water Control

Slide Series Number	Tooth Number	Method of Exposure	Reparative Dentin Formation	Reaction of Pulp
30 Days Postoperative				
7985	28	Exp	Sml	USat
7999	2	#2	Sml	USat
8005	9	Exp	Mod	Sat
8012	18	Exp	None	Nec
8018	26	Exp	None	Nec
8019	27	Exp	None	Nec
8021	30	Exp	Lge	Sat
90 Days Postoperative				
8024	3	#2	Mod	Sat
8026	5	#2	None	Nec
8030	9	Exp	Lge	Sat
8032	11	1J	Lge	Sat
8033	12	Exp	None	Nec
8037	19	Exp	Sml	USat
8045	26	Exp	Lge	Sat

TABLE VII

Response of the Pulps to Each Medicament
at Each Time Interval

Pulp Capping Agent	Length of Treatment	Response of Pulp: Satisfactory	Unsatisfactory	Necrosis
VSH	30 days	6	1	0
VSH	90 days	7	0	0
VSW	30 days	3	4	0
VSW	90 days	7	0	0
SW	30 days	2	2	3
SW	90 days	4	1	2
SH	30 days	2	1	4
SH	90 days	4	2	1

TABLE VIII

Comparison of All Teeth Treated with Each Medicament

Pulp Capping Agent	Response of the Pulp in Per Cent:		
	Satisfactory	Unsatisfactory	Necrosis
VSH	92.9	7.1	0
VSW	71.5	29.5	0
SW	42.9	21.4	35.7
SH	42.9	21.4	35.7

TABLE IX

Statistical Analysis of Variance of Reparative Dentin Formation

Variation	Degree Freedom	Mean Square+	F
Between Pulp Capping Agents	3	64482.83	4.84**
Between 30 Days and 90 Days	1	30504.50	2.29 n.s.
Between Pulp Capping Agents and Time	3	5603.50	0.42 n.s.
Theoretical Error		13333.33	

Statistical Analysis of Variance of Pulp Reactions

Variation	Degree Freedom	Mean Square+	F
Between Pulp Capping Agents	3	82256.33	6.17**
Between 30 Days and 90 Days	1	69938.00	5.25*
Between Pulp Capping Agents and Time	3	15224.33	1.14 n.s.
Theoretical Error		13333.33	

+ These are mean squares of transformed data.
** Statistically significant, $P<.005$
n.s. Not significant
* Statistically significant, $P<.025$

TABLE X

Statistical Comparison of Each Pulp Capping Agent with One Another

Reparative Dentin Formation:

	Z Score
SW vs. SH	1.868 n.s.
SW vs. VSW	4.995**
SW vs. VSH	6.886**
SH vs. VSW	6.863**
SH vs. VSH	8.753**
VSW vs. VSH	1.890 n.s.

Reaction of the Pulp:

	Z Score
SW vs. SH	
SW vs. VSW	4.869**
SW vs. VSH	9.704**
SH vs. VSW	4.869**
SH vs. VSH	9.704**
VSW vs. VSH	4.835**

** - Statistically significant, P<.01
n.s. - Not significant
SW - Starch and water
SH - Starch and hyaluronidase
VSW - Vancomycin, starch and water
VSH - Vancomycin, starch and hyaluronidase

TABLE XI

Results of Statistical Comparison of Each Pulp Capping Agent with One Another in a Tabulated Form for:

Reparative Dentin Formation:

	SH	VSW	VSH
SW	n.s.	**	**
SH		**	**
VSW			n.s.

Reaction of the Pulp:

	SH	VSW	VSH
SW	n.s.	**	**
SH		**	**
VSW			**

n.s. - Not significant
** - Statistically significant, $P<.01$
VSW - Vancomycin, starch and water (Group I)
VSH - Vancomycin, starch and hyaluronidase (Group II)
SW - Starch and water (Group III)
SH - Starch and hyaluronidase (Group IV)

Figure 1. Implants in place immediately after surgery.

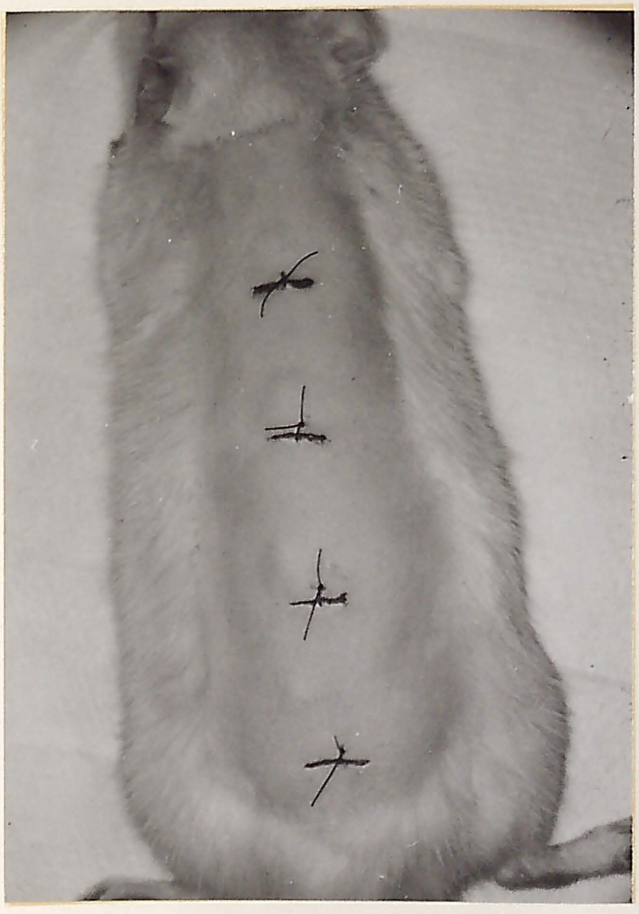
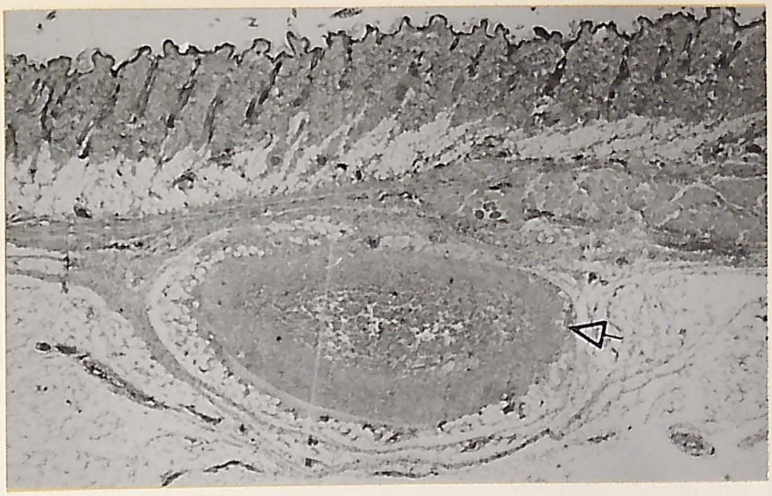


Figure 2. Cavity preparations and pulp exposures in an experimental monkey.

Figure 3. Photomicrograph of a section through a subcutaneous implant of vancomycin, starch, and hyaluronidase at two days demonstrates a mild response. Arrow indicates implant material. (Hematoxylin and eosin stained. Original magnification X 40).

Figure 4. A section through a 16 day implant specimen of vancomycin, starch, and water indicating a mild response. Arrow indicates implant material. (Hematoxylin and eosin stained. Original magnification X 40).

Figure 5. Thirty-two day implant indicating a mild response. Arrow indicates implant material of starch and water. (Hematoxylin and eosin stained. Original magnification X 40).



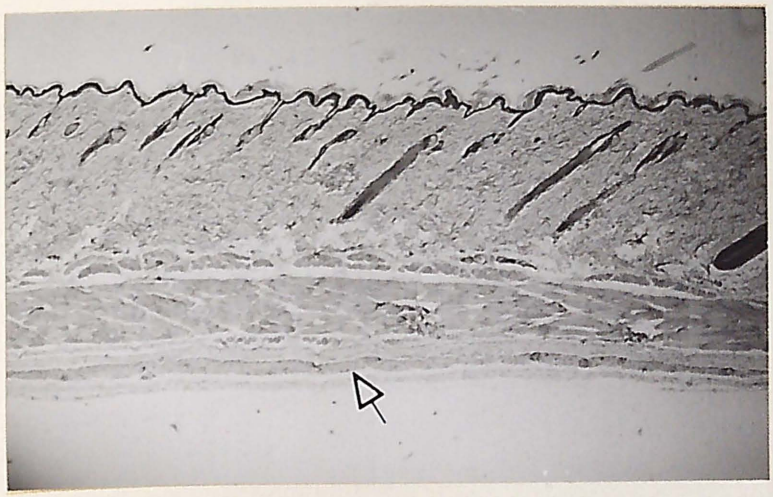


Figure 6. Unsatisfactory response in a maxillary left first bicuspid treated for 30 days with starch and hyaluronidase. Note the lack of reparative dentin and the numerous inflammatory cells near the dentin chips. (Hematoxylin and eosin stained. Original magnification X 40).

Figure 7. Mandibular right second molar treated with vancomycin, starch and water for 30 days. The absence of inflammatory cells and coalescence of reparative dentin around the chips indicates a satisfactory response.

A. Artifact resulting from poor fixation in this area.

B. Artifact resulting from a separation of odontoblasts from the canal wall.

(Hematoxylin and eosin stained. Original magnification X 40).


A
B

Figure 8. A satisfactory response in a mandibular right second molar treated for 90 days with vancomycin, starch and hyaluronidase. Artifacts in this section are the result of odontoblasts separating from the canal walls and reparative dentin. Note the "growing out" of reparative dentin into the cavity preparation (arrow). (Hematoxylin and eosin stained. Original magnification X 40).

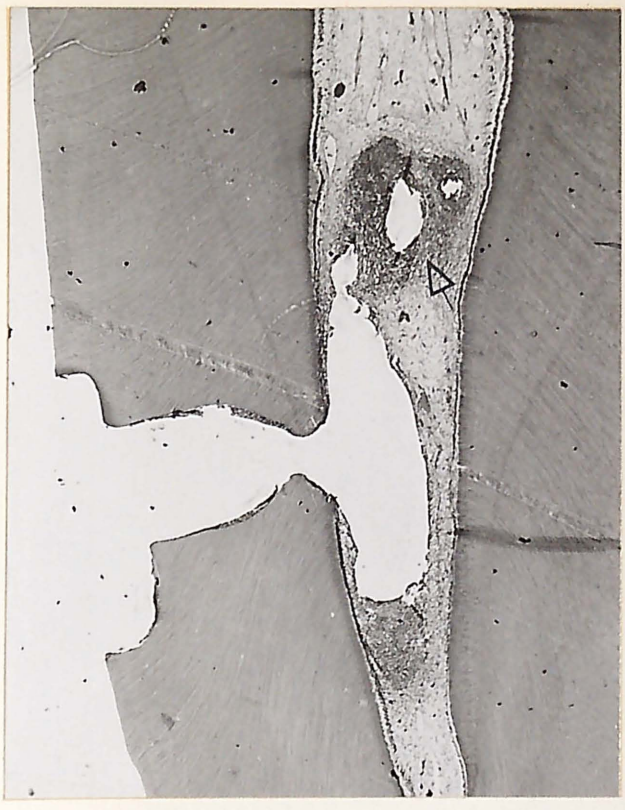
Figure 9. Unsatisfactory response in a mandibular right central incisor treated for 30 days with vancomycin, starch and water. A coronal abscess is present (arrow). (Hematoxylin and eosin stained. Original magnification X 40).

Figure 10. Microscopic section adjacent to section in Figure 9. Note the bacteria scattered in the area of the abscess and the dark staining particles of starch that have penetrated into the pulp.

A. Abscess

B. Starch particles

(Brown and Brenn stained. Original magnification X 40).




B
A

Figure 11. Necrotic pulp in a mandibular left lateral incisor treated for 30 days with starch and hyaluronidase. Note the dentin chips in the canal and lack of reparative dentin. (Hematoxylin and eosin stained. Original magnification X 40).

Figure 12. Unsatisfactory response of a maxillary left first molar treated for 30 days with vancomycin, starch and hyaluronidase. Note the minimal amount of reparative dentin around the dentin chips. Inflammation was lacking in the area of the dentin chips. (Hematoxylin and eosin stained. Original magnification X 40).

Figure 13. An unsatisfactory response in a maxillary right first bicuspid treated for 90 days with starch and water.
A. Necrotic area.
B. Extent of bacterial penetration.
C. Pyogenic membrane.
D. Chronic inflammation.
(Hematoxylin and eosin stained. Original magnification X 40).

Figure 14. Section adjacent to section in Figure 13. Note clusters of bacteria in coronal area (arrow). (Brown and Brenn stained. Original magnification X 40).


A
B
C
D

Figure 15. A satisfactory response of a maxillary right central incisor treated for 30 days with vancomycin, starch and water. Note the large amounts of reparative dentin on the canal walls and a dentin chip. (Hematoxylin and eosin stained. Original magnification X 40).

Figure 16. A satisfactory response of a maxillary right central incisor treated for 90 days with vancomycin, starch and water. Compare pronounced reparative dentin formation with that in Figure 15. (Hematoxylin and eosin stained. Original magnification X 40).

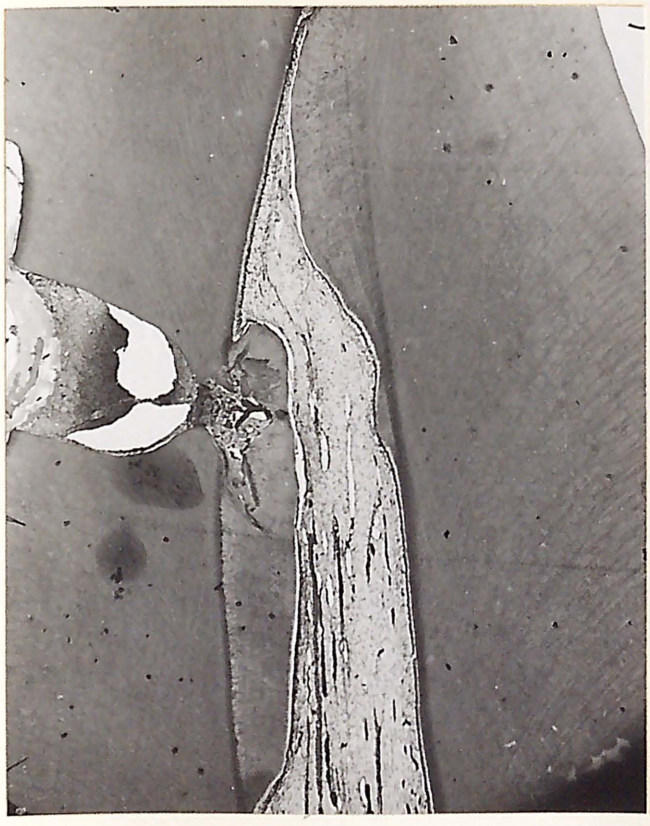
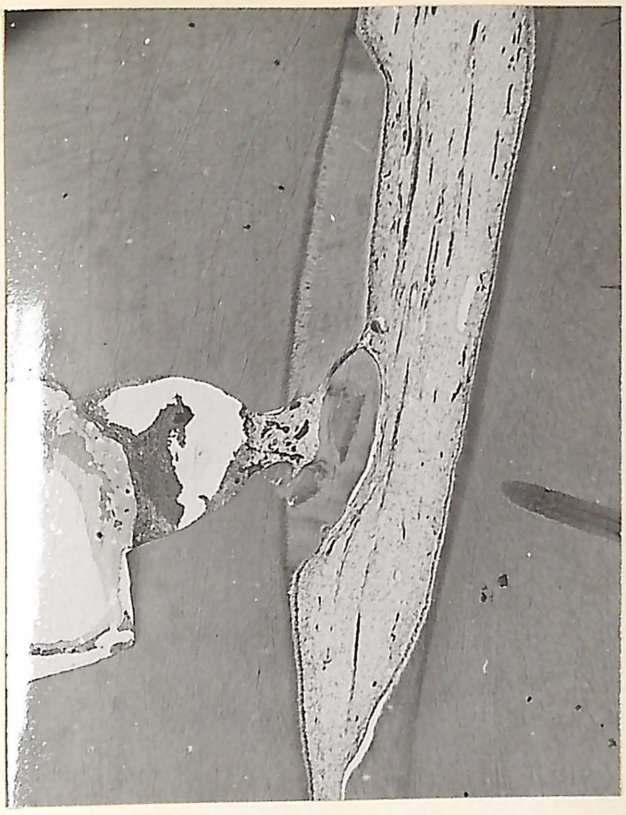
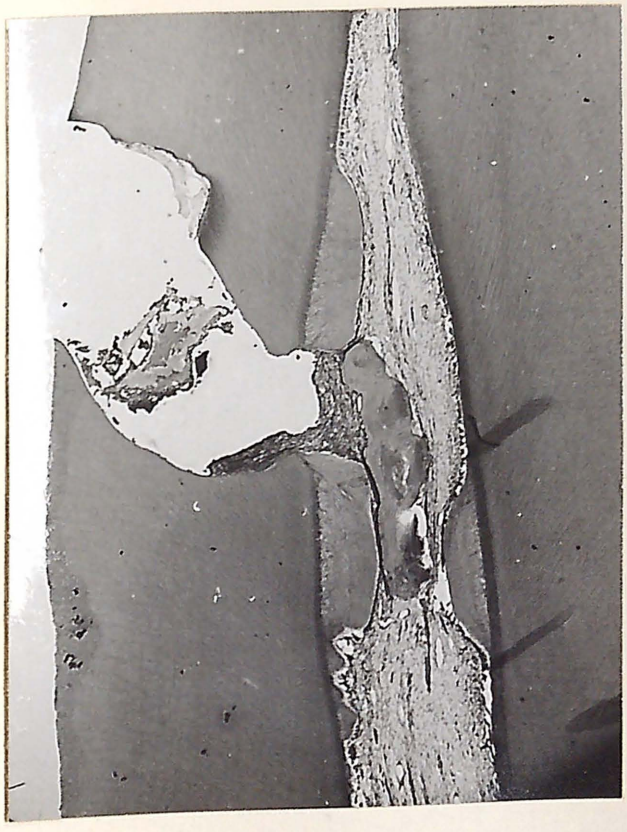


Figure 17. A satisfactory response in a maxillary left central incisor treated for 90 days with starch and water. (Hematoxylin and eosin stained. Original magnification X 40).

Figure 18. A satisfactory response in a mandibular left central incisor treated for 90 days with vancomycin, starch, and hyaluronidase. Note the reparative dentin on the canal wall opposite the exposure. (Hematoxylin and eosin stained. Original magnification X 40).





DISCUSSION

In this study, as in a study by Baker,[33] it was demonstrated that the pulps of monkeys exposed to the oral environment for 24 hours become contaminated with primarily gram positive organisms. The role of these bacteria in pulp pathosis has been identified by Kakehashi, Stanley and Fitzgerald.[52] They exposed the pulps of molars of 24 germ-free and 15 conventional rats and left them open to the oral environment for from one to 42 days. After eight days or longer the pulps of conventional rat molars exhibited complete necrosis, while 18 of the 24 germ-free rat molars exhibited dentin bridging and little inflammation.

The antibiotic used in this study has been used effectively in treating oral infections by Mitchell and Holmes,[53] Mitchell *et al.*[54] and Hood and Collins.[55] This suggests that vancomycin might be useful in treating pulps contaminated with bacteria from the oral cavity. In a preliminary part of their investigation, the experimental pulp capping agents containing 10 per cent vancomycin were effective against all gram positive organisms tested. Other investigators[55-59] concur with this finding of sensitivity of gram positive organisms to vancomycin.

Local inflammatory reactions to vancomycin have been reported.[60] The pulp capping agents used in this

study were screened by a method devised by Mitchell.[61] Histologic evaluation of subcutaneous implants in rats revealed mild responses to all test medicaments.

Kutscher and Yigdall[62] found the activity of antibiotics is often decreased when the antibiotics are combined with other therapeutic agents. In this study the activity of vancomycin was not affected when mixed with the components of the experimental agents. Hyaluronidase, one of the therapeutic agents tested by Kutscher and Yigdall, did not in their study, or in this study, affect the activity of the antibiotics.

Hyaluronidase increases extravascular flow and reduces edema. Edema within the confines of the canal walls has been of concern.[63] Enlargement is impossible within the canal walls unless an exposure or necrotic area provides space in which edema may occur. Lewin-Epstein and Silbermann[64] used hyaluronidase in the treatment of injured pulps in rats. Histologic examinations revealed that more successful recovery resulted when hyaluronidase was used when compared to a control lacking hyaluronidase. In this study, when there was doubt as to a satisfactory or unsatisfactory response, the response was recorded as unsatisfactory.

Satisfactory responses were observed in all but one of the infected pulps treated with vancomycin, starch

and hyaluronidase. The unsatisfactory response observed was in the 30 day animal (Figure 12). No inflammation was observed, but a suspicious area which may have been an abscess and a minimal attempt at reparative dentin formation was seen. Fiore-Donno and Baume[31] stressed the importance of a solid dentin bridge in pulp healing to prevent reinfection, and in this study the amount of reparative dentin observed in a tooth influenced its final evaluation. On the other hand, one of the 90 day specimens treated with both vancomycin and hyaluronidase (Figure 8) responded with excessive amounts of reparative dentin including reparative dentin in an area which had been prepared with a bur. This dentin appeared different from that found on the canal walls and around dentin chips. It resembled bone rather than the irregular appearance of reparative dentin. This unusual appearance was due to the entrapped odontoblasts within this dentin resembling lacunae of bone. Perhaps the one tooth judged unsuccessful would have demonstrated a more satisfactory result after a longer period of time since no inflammation was observed.

Vancomycin, starch, and water produced satisfactory responses in 71.5 per cent of the treated teeth. The 30 day treatments responded with four unsatisfactory results, while the 90 day treatments all responded in a favorable

manner. One responded with excessive reparative dentin similar to that observed in a tooth (Figure 8) treated with vancomycin, starch, and hyaluronidase. Since the unsatisfactory responses were observed only in 30 day specimens, one can speculate as to whether a more favorable response would have resulted in these teeth after 90 days.

All of the teeth treated for 90 days with vancomycin in any form responded well and none of these vancomycin treated teeth were necrotic at either 30 or 90 days.

Hyaluronidase, when used with vancomycin, seems to affect responses at 30 days but not at 90 days when compared to responses to vancomycin alone. The one unsatisfactory response when hyaluronidase was used with the antibiotic was evaluated as unsuccessful not because of inflammation, but because of minimal reparative dentin formation and a questionable area near the exposure.

Necrosis occurred in 35.7 per cent of the teeth in both control groups, (i.e., teeth treated with starch and water and those treated with starch and hyaluronidase). There was a difference in the response of control teeth in each animal. Seven of the 14 control teeth in the 30 day animal became necrotic, while only three of the 14 teeth in the 90 day animal became necrotic. This

same pattern held true for teeth treated successfully with control medications. Eight of the 90 day control teeth were treated successfully while only four of the 30 day control teeth responded well.

The pulps of the 90 day animal responded more favorably to all treatments when compared to the pulps of the 30 day animal. This was found to be statistically significant and may be related to several factors, such as (1) difference in the inherent ability of each animal to resist pulpal infection; (2) variations in the pathogenic organisms present in the oral flora of each animal; and (3) variations in the ability of the investigator to duplicate procedures in each animal — since they were operated on different days. Owing to the tedious nature of this experimental procedure, it is possible that some pulps were traumatized more extensively than others during the mechanics of exposing and capping. A suggestion for future studies of this nature would be the use of vital dyes in the long term animals at times corresponding to the sacrifice of the short term animals. These dyes may permit a more accurate comparison between animals.

Several investigators[11,13,33,52,65] have noted a relationship between the presence of inflammation and the amount of reparative dentin present. A similar ob-

servation was made in this study; inflammation seemed to inhibit reparative dentin formation, with the more severely inflamed pulps producing the least amount.

Complete bridging was not found in any of the teeth treated in this study. More bridging was observed in the 90 day specimens than in the 30 day specimens, which may be explained by the hypothesis that dentin bridging is a progressive activity which may take longer than 90 days for completion.

The successful response observed in 42.9 per cent of the control teeth treated with starch and water and starch and hyaluronidase may be indicative of an inherent resistance of the animals to pulp infection, or it may be that these pulps did not become infected after they were exposed and left open to the oral environment. No bacteria were observed in a specially stained section of a successfully treated control tooth which lends support to the latter statement. Perhaps in future studies the pulps should be left open to the oral environment longer than 24 hours.

One problem of _in vivo_ studies of the pulp and microorganisms is that of reinfection of the pulp following the original innoculation and treatment. A similar problem occurred in a study of this kind by Walshe[65] in which he sealed the cavities after treatment with zinc

oxide and eugenol. He suggested that in future studies a layer of amalgam be placed over the zinc oxide and eugenol, thus giving a closure technique with both the sealing properties of zinc oxide and eugenol[66] and the resistance to abrasion of amalgam. This technique was incorporated in this study and the results indicate that it is adequate to overcome this problem.

While reinfection was not a problem in this study, the initial innoculation of exposed pulps may have been, since a number of control teeth expected to be infected, showed no evidence of microorganisms. A number of factors that could cause this problem were noted earlier, but the author believes that his technique in innoculating the pulps was not one of them.

The oral flora of the monkey has been demonstrated to be similar to that of man.[67,68] A hypothesis of this study is that the monkey will respond to antibiotic therapy in a manner similar to man.

SUMMARY AND CONCLUSIONS

Studies of pulp capping agents composed of antibiotics alone or antibiotics in combination with other therapeutic agents were described in the literature reviewed. Most of these reports were inconclusive or contradictory in nature. This modified double blind controlled study was initiated to investigate the use of a potent antibiotic, vancomycin hydrochloride, by itself and in combination with an anti-inflammatory enzyme, hyaluronidase, as a pulp capping agent in intentionally infected pulps of the Macaca Speciosa monkey.

Preliminary investigations revealed the effectiveness of the antibiotic containing agents against gram positive organisms and the mild reaction of the tissues to their presence. Pulps of four teeth exposed to the oral environment for 24 hours were specially stained (Brown and Brenn) to demonstrate the presence of bacteria. Microscopic examination revealed the presence of many organisms in the area of the exposures in these teeth. The major role played by bacteria in pulp pathosis was discussed.

The pulps of 56 permanent teeth in two monkeys were mechanically exposed to the oral environment for 24 hours in the principal portion of this study. Each of these pulps was then covered with one of the four experimental pulp capping agents. Each medicament was placed in 14 teeth. The experimental drugs were composed of vancomycin, starch and

hyaluronidase; vancomycin, starch, and water; starch and water; and starch and hyaluronidase. These medications were sealed in the preparations with zinc oxide and eugenol with a final filling of amalgam placed over this cement.

The teeth were extracted prior to sacrificing, one animal at 30 days and the other animal at 90 days. The teeth were decalcified, serially sectioned, and stained for histologic evaluation.

Among the teeth treated with vancomycin, starch, and hyaluronidase, all but one (92.9 per cent) responded in a satisfactory manner with moderate to large amounts of reparative dentin and little inflammation. The majority of the teeth treated with vancomycin, starch, and water (71.5 per cent) responded with large or moderate amounts of reparative dentin and little inflammation. None of the teeth treated with either vancomycin, starch, and hyaluronidase, or vancomycin, starch, and water became necrotic. Both groups of teeth treated with control medications, starch and water and starch and hyaluronidase, had similar responses to each other. Pulpal necrosis was present in 35.7 per cent of the teeth in each control group and another 21.4 per cent of each control group responded in an unsatisfactory manner with little or no reparative dentin formation and the presence of many inflammatory cells in the coronal pulp. Forty-two and nine tenths per cent of each

control group responded with moderate to large amounts of reparative dentin formation and little inflammation.

Statistically, vancomycin, starch, and hyaluronidase and vancomycin, starch, and water were significantly more effective as pulp capping agents in preventing inflammation and promoting reparative dentin formation when compared with starch and water and starch and hyaluronidase. With respect to pulpal reaction, vancomycin, starch, and hyaluronidase resulted in significantly more favorable responses than vancomycin, starch, and water.

In seven teeth studied histologically for the presence of bacteria, microorganisms could be demonstrated in the pulps of two of three teeth showing chronic inflammation or abscess formation. The presence of bacteria could not be demonstrated in four successfully treated teeth.

From the results of this investigation, the following conclusions were made:

1. Vancomycin-containing pulp capping agents are effective in controlling infection and in promoting reparative dentin formation in monkey pulps.
2. Hyaluronidase, an anti-inflammatory enzyme, was of some additional benefit when used in combina-

tion with the antibiotic; however as the length of treatment increased from 30 to 90 days the value of the enzyme became less apparent.

3. The encouraging results indicate the need for further investigations using pulp capping agents containing this antibiotic and anti-inflammatory enzymes; furthermore, a clinical study is indicated.

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ABSTRACT

Treatment Of Infected Dental Pulps Of Monkeys With Vancomycin And Hyaluronidase

By
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This study was undertaken to investigate histologically the effect of a combination of an antibiotic and an anti-inflammatory enzyme when used as a medication in direct pulp therapy.

The pulps of 56 teeth in two Macaca Speciosa monkeys, exposed and left open to the oral environment for 24 hours to insure contamination, received direct treatment with one of four experimental medications: (1) vancomycin, starch, and hyaluronidase; (2) vancomycin, starch, and water; (3) starch and water; and (4) starch and hyaluronidase. At 30 days the teeth were removed from one animal and at 90 days from the other for histologic interpretation.

A satisfactory response was observed in 92.9 per cent of the teeth treated with vancomycin, starch, and hyaluronidase; in 71.5 per cent of the teeth treated with vancomycin, starch, and water; and in 42.9 per cent of the teeth treated with both starch and water and starch and hyaluronidase. None of the teeth treated with vancomycin, starch, and water and vancomycin, starch, and hyaluronidase became necrotic, while 35.7 per cent of the teeth treated with starch and water or starch and hyaluronidase became necrotic.

Under the conditions of this investigation, vancomycin containing pulp capping agents are effective in controlling infection and in promoting reparative dentin formation in monkeys. The benefit of hyaluronidase when used in combination with vancomycin was questionable.